THE NORMAL GROWTH AND RESPIRATION OF TETRAHYMENA GELEII

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The early population growth phases of *Tetrahymena geleii* (Furgason, 1940) are well established due to the work of Elliott (1933), Phelps (1935), and Hetherington (1936). The later growth phases are relatively unexplored. Phelps (1935, 1936) outlined the effect of food concentration and of the age of the inoculum. He found that Buchanan's (1918) lag phase, exponential phase, decreasing acceleration phase, and stationary phase were present in the growth of bacteria-free cultures of this ciliate and unlike most bacterial populations the stationary and lag periods were not influenced by the size of the inoculum. Varying the size of the inoculum varied only the length of the exponential phase. He demonstrated that within wide limits the concentration of food had no effect on the rate of growth in the exponential phase but only on the maximum concentration.

No complete explanation for the appearance of these various population growth phases is yet available. Food certainly plays a part, although by no means is it the only agent. Since Phelps found that in yeast autolysate concentrations between 0.4 per cent and 20 per cent there is a direct proportion between the food concentration and the maximum yield of ciliates, any factor such as Bail's (1929) "population pressure" or the presence of toxic excretory products would seem to be ruled out. Monod (1935) was likewise unable to demonstrate the appearance of any effects due to toxic substances. Kidder (1941b) suggests that an inhibitory substance as well as an accelerative factor may be produced as a result of a series of experiments on growth in "biologically conditioned" media. The oxygen tension has an effect on the length of the exponential phase and also on the maximum yield (Phelps, 1935) while the carbon dioxide tension apparently is not a limiting factor in cultures of *Tetrahymena geleii* (Jahn, 1936). The pH of the medium can influence the growth as shown indirectly by the respiration

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measurements of R. H. Hall (1941) and directly by numerous authors including Elliott (1933), Johnson (1935) and Kidder (1941a).

There has been no thorough examination of the relationships existing between population growth and cell growth in the various growth phases of protozoan cultures. These relationships become important in respiration and metabolic studies in which the information needed is the activity per unit of protoplasm rather than per organism. A study has been made therefore of the relationships existing between cell growth and population growth. This data has been correlated with normal oxygen consumption in the various population-growth phases.

It will be shown that changes in cell size may occur during the lag and early exponential population-growth phases depending on the conditions of the experiment and that consequently there may be an increase in total protoplasm due to cell growth rather than combined cell and population growth. It will likewise be shown that characteristic cell-size changes can be correlated with other population growth phases. The behavior of populations in very old cultures will be briefly noted.

This work was done at the Arnold Biological Laboratory, Brown University, and the Marine Biological Laboratory, Woods Hole, Massachusetts. I wish to express my sincere appreciation to Dr. George W. Kidder for his helpful suggestions and sustaining interest.

Material and Methods

The organism used in these experiments was *Tetrahymena geleii* (Hetherington strain). In the experiments on the early growth phases cultures were grown in 250 ml. Kidder culture flasks (Kidder, 1941b) in 100 ml. lots of 2 per cent Difco proteose peptone made up with Pyrex-distilled water. Cultures used in the work on size and population changes over long periods of time were grown in liter flasks using 500 ml. of culture fluid.

All cultures were grown in an incubator at 26° ± 0.3° C. and under sterile conditions. Sterility was checked by inoculation onto nutrient agar slants and Petri dishes which were incubated at 26° ± 0.3° C. for two weeks before a final diagnosis of sterility was made. All contaminated cultures were discarded. In the work on population and size changes over long periods the cultures were checked after each withdrawal of a sample, and at the end before the flasks were discarded.

Population counts were made by direct counting of the live organisms following serial dilution in 2 per cent proteose peptone until the concentration of the ciliates was between 25 and 125 organisms per 0.2 ml. This amount was then streaked out on glass slides and counted with a

binocular dissecting microscope. The error involved in this method amounts to ± 5 per cent.

In the work on size changes the organisms were killed by fixation in 5 per cent formalin and measured with an ocular micrometer. No appreciable shrinkage due to the formalin was observed.

Dry weight measurements were made by centrifuging the suspension of organisms, removing the supernatant and washing three times with distilled water. Ten ml. of the final suspension were then pipetted into a porcelain crucible which was placed in an oven at 100° ± 0.5° C. for 24 hours. They were then removed to a desiccator until the weights became constant.

Nitrogen determinations of washed organisms were made by the micro-Kjehldahl method. The apparatus used is described by Pregl in Roth's (1937) translation. The method used was that of Bang (1916), Parnas and Wagner (1921) as modified by Elek and Sobotka (1926).

Respiration measurements were made with the standard Barcroft-Warburg apparatus. The technique employed is outlined in Dixon (1934). Vessels of about 10 ml. capacity were used with a total of 2 ml. of fluid in each. Carbon dioxide absorption was by means of a roll of Whatman (starch-free) No. 40 filter paper soaked with 0.2 ml. of 20 per cent KOH. The apparatus was set to give 140 oscillations per minute with a stroke of 4 cm. Temperature in all determinations was maintained at 26.8° ± 0.1° C.

The suspensions of organisms used in respiration determinations were made up of organisms resuspended in a 0.005 M phosphate buffer of pH 6.9. The pH was checked at the end of each experiment. The density of the suspensions used was between 100,000 and 500,000 ciliates per ml. Within these limits neither the rate of shaking nor the amount of potassium hydroxide was a limiting factor in the determinations of oxygen consumption. Oxygen consumption is expressed as cubic millimeters of dry gas under standard conditions of temperature and pressure.

Experimental Results

Normal Growth

The normal population growth curves obtained were similar to those obtained by Phelps (1935, 1936) and Kidder (1941a). Cultures were set up using 2 per cent proteose peptone in Kidder culture flasks as previously described. A normal curve (*A*) is shown in Figure 1. The lag period is not shown. The interdivisional period during exponential

growth was 3.32 hours for this set of experiments. Population counts were obtained by using as the diluent conditioned 2 per cent proteose peptone in which ciliates had been grown for the same length of time as those in the experimental flask. The organisms were killed by gentle heating in a flame, centrifuged out, and the supernatant used as the diluent. Following dilution of a sample of the experimental culture the dilutions were allowed to stand for ten minutes following which the counts were made. When a fresh 2 per cent proteose peptone was used as the

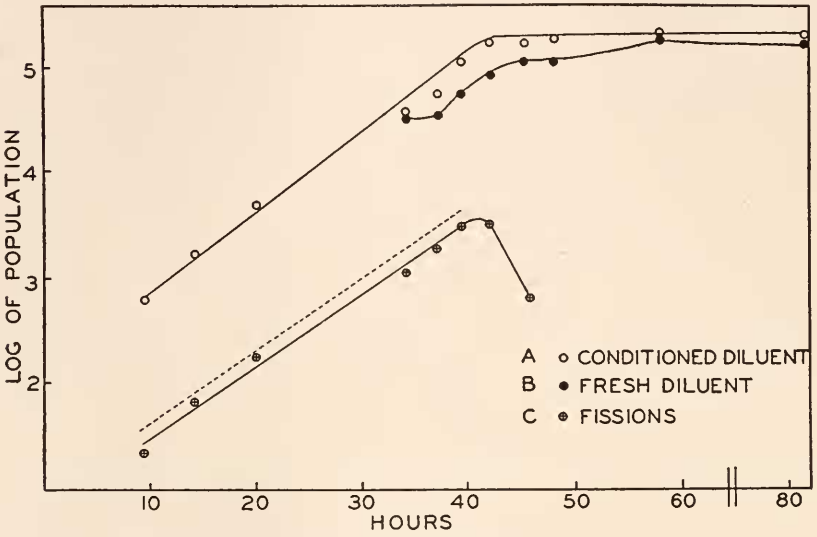

Fig. 1. Population and fission curves in 2 per cent proteose peptone. The dotted line represents the theoretical fission curve. All curves are on the same ordinate.

diluent, curve *B* was the result. This curve indicates the presence of an increased sensitivity of the organisms as the population approaches the end of the phase of exponential growth. This sensitivity was not apparent in the early exponential phase and gradually disappeared in the hours following the end of the exponential phase. It was later found that accurate counts (5 per cent) could be made using fresh proteose peptone as the diluent if the diluting and counting procedure were completed within five minutes.

The average fission time will of course vary with the criteria of the investigator. In this study fission was arbitrarily assumed to have begun when the first external indication of the cytokinesis could be observed using a dissecting binocular microscope with 10 × oculars and

10 × objectives. The average of 20 determinations of fission time at room temperature and in hanging drop preparations was 12.5 minutes. Curve *C* in Figure 1 represents the logarithm of the number of fissions in the exponential phase and in the hours immediately following it. The dotted line in Figure 1 represents the theoretical fission curve during the exponential phase.[2] As would be expected, the rate of increase of fissions is constant. The results are summarized in Table 1.

TABLE 1

Fission rate compared with the rate of population growth. Eight experiments. The theoretical number of fissions during the first 40 hours is 0.044 per cent.

Time in hrs.	Population/ml.	Fissions/ml. experimental	Per cent fissions experimental
10	594	22	0.038
14	1,585	63	0.040
20	4,740	168	0.038
34	30,600	1,060	0.035
37	53,100	1,780	0.034
40	89,100	2,982	0.034
43	141,300	3,160	0.022
46	149,700	595	0.004
49	173,800	—	—
59	211,400	—	—
82	210,000	—	—

It will be noted from Figure 1 that the percentage of fissions drops very rapidly when exponential growth ceases. After 46 hours the percentage of fissions was only 0.004. Beyond this point the number was so low that examination of 40,000 to 50,000 ciliates would not show enough fissions to form an accurate percentage figure. This indicates that in the stationary phase there is negligible death occurring. The growth rate of practically zero does not represent a balance between an increase due to fission and a decrease due to death of some organisms but an actually stationary population.

This conclusion is reinforced by a consideration of Figure 2, the data for which are contained in Table 2. Each length given in Table 2 repre-

[2] The population growth during the exponential phase may be described by the equation (1) $N = N_0 e^{\lambda G}$ where N = the number of individuals per unit volume, $\lambda = \log_e 2$ and G = the interdivisional period. The number of organisms at any time in the exponential phase *exclusive* of those in fission may be described by (2) $N' = N_0 e^{\lambda[G-S]}$ where S = the average time occupied in fission by each organism. Subtracting (2) from (1), and rearranging in terms of G, the percentage of individuals in fission at any time during the exponential phase is (3) $\frac{2^{S/G} - 1}{2^{S/G}}$.

Table 2

Cell growth compared with population growth. Size data from 14 experiments; each point represents average of 150 measurements. Weight determinations each represent average of three or more determinations. The MC signifies the end of the period of exponential growth.

Time in hrs.	Population/ml.	Length in micra	Time in hrs.	Wt. in mgm. per 10^6 organisms
0	100	74	10	5.32
4	120	68	MC	—
11	1,000	58	10	5.85
16	2,510	53	20	7.34
26	31,600	53	30	8.01
MC	—	—	40	—
3	123,200	57	50	8.34
19	135,000	70	60	8.79
26	158,600	73	70	8.82
40	162,500	75	80	—
75	174,300	75	90	8.85
100	128,800	75	130	8.55[a]
130	120,000	75	191	9.04[b]
145	102,700	71	—	—
169	100,100	70	—	—
191	89,200	69	—	—

[a] One determination. [b] Two determinations.

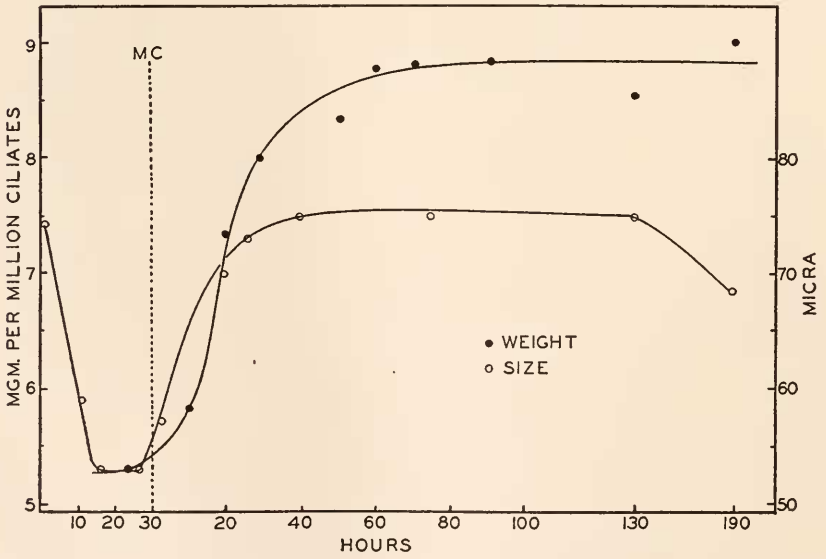

Fig. 2. Size and weight changes in 2 per cent proteose peptone flask cultures of *Tetrahymena geleii*. The abscissa represents the age of the culture.

sents an average of 150 measurements. The length was taken as an adequate criterion of the size, since the ratio of length to width did not vary significantly over the time range examined. The weight measurements are the average of 36 determinations and are plotted as the average of 10-hour intervals. Data for both weight and size are translated into terms of a 30-hour exponential period for purposes of comparison, and all determinations made after the exponential phase were calculated with the end of that period as the point of departure. Data such as these are significant in terms of the age of the culture with respect to the growth

TABLE 3

Nitrogen determinations. Three determinations were made on urethane-treated organisms in a separate experiment not reported here.

Dry wt. in mgm. of organisms	N in mgm.	Per cent N	Age of organisms in terms of 30 hour MC
27.1	3.1	11.4	23 hours
67.5	4.8	7.1	84 hours
62.6	6.5	10.4	93 hours
64.6	7.3	11.3	110 hours
22.1	2.2	10.0	127 hours
27.3	2.9	10.6	40 hours
12.9	1.3	10.8	47 hours[a]
14.0	1.6	11.4	34 hours[b]
71.7	7.7	10.7	87 hours
12.4	1.4	11.3	59 hours[c]
	Average	10.5	

[a] 13 hrs. in 1 per cent urethane.
[b] 11 hrs. in 1 per cent urethane.
[c] 25 hrs. in 1 per cent urethane.

phases and not in terms of absolute time units. With different-sized inocula the length of the exponential phase in these experiments varied from 15 to 45 hours. All weight measurements made on exponential phase organisms, when translated into terms of a 30-hour exponential growth period, came at the same place in time, e.g., 25 hours. Since they all agreed very closely it is clear that the weight of the individual ciliates is quite constant during the exponential phase. The time relations in the increase in size and weight following the end of the period of exponential population growth were comparable, regardless of the length of the exponential period. This demonstrates that size and weight measurements on organisms such as these must be judged with relation to the normal population curve and not by an absolute time scale.

Nitrogen determinations (Table 3) indicate a constant nitrogen per-

centage of 10.5 per cent. In other words, the increase in weight shown in Figure 2 represents an increase in protoplasm and not merely an increase in stored carbohydrate or something of like nature. The average nitrogen content agrees well with the scanty data available. Panzer (1911), in an analysis of the coccidian *Gaussia gadi*, found the nitrogen to be 9.7 per cent of the dry weight. Hutchens (1941a) reports the nitrogen content of *Chilomonas paramecium* to be 10 to 11 per cent.

In the lag and early exponential phases the shape of the size curve in Figure 2 depends upon the size of the ciliates used for inoculation of the culture. If organisms 50 to 75 hours old are used they are usually about 75 μ long. Upon inoculation into fresh media and the onset of division, the average size progressively decreases until a constant size of around 53 μ is reached. This size is then maintained to the end of the exponential period. On the other hand if organisms from a 30- to 40-day old culture (about 35 μ long) are used for the inoculation, there is an *increase* in average size until the constant size of about 53 μ is reached. Thus it is clear that in some cases at least the changes in individual size preceding and during cell division must be interpreted in relation to the age of the organism.

Population changes were followed for periods up to 90 days and cell-size changes up to 45 days in 2 per cent proteose peptone cultures. In cultures in which the *MC* occurred at 36 hours, the stationary phase of population growth persisted for five days, following which a phase of declining population set in. This phase was sometimes marked by rather sudden increases in population although the overall picture was one of gradually decreasing population. It is clear that the latter parts of Buchanan's classical population curve are not present in cultures of this type and in the time range examined. There is no clear phase of accelerated death or exponential death and the population decrease that is observed is not necessarily an even decline.

Cell size in cultures of this type, after increasing to a maximum of 75 μ in length as shown in Figure 2, maintained the maximum size for four days after which there was a gradual decrease. In three experiments this decrease in size continued for 34 days at which point there was no further change for six days when the experiments were discontinued. The ciliates had decreased to 36 μ length in these 40-day old cultures.

Normal Respiration Rates

Table 4 gives the results of normal respiration measurements on ciliates in 0.005 M phosphate buffer and in a 2 per cent proteose pep-

tone medium buffered with 0.005 M phosphate. The Q_{O_2} is defined as cu. mm. O_2/hr./mgm. dry weight of tissue. The exponential phase ciliates were taken between 20 hours and 30 hours from cultures in which exponential growth would have ceased at 30 hours. The stationary phase organisms were taken at around 50 hours from similar cultures.

Provided the concentration of organisms in the suspension measured is above the maximum concentration obtained in a normal culture, the

TABLE 4

Normal respiration values. The figures in parentheses refer to the number of determinations made. The Q_{O_2} represents cu. mm. O_2/hr./mgm. dry weight of ciliates.

	Exponential phase organisms		Stationary phase organisms	
	In 0.005M PO_4 buffer (65)	In 2% proteose peptone (11)	In 0.005M PO_4 buffer (21)	In 2% proteose peptone (14)
Q_{O_2}	26.0	81.1	16.5	78.8
Cu. mm./hr./10^6 ciliates	140.2	433.0	133.7	633.0

oxygen consumption is a linear function of time over a period of three hours in all cases. Experiments were terminated at three hours.

It might be expected that exponential phase ciliates would show a higher rate of respiration than organisms in the stationary phase. This difference does appear, particularly in buffer suspensions.

DISCUSSION

Very little satisfactory work correlating population with size changes has been done with protozoa although there are good data on bacteria and yeasts. Henrici (1928) showed that cells of *Bacillus megatherium* increase in size during the lag period and up to the point at which population growth rate is maximum, whereupon they begin to decrease in size immediately. Huntington and Winslow (1937), in an examination of the size changes in *Escherichia coli, Salmonella gallinorum* and *Salmonella pullorum,* show that in all three there is an increase in size in the lag period and a decrease in size during the exponential period. Richards (1928) has shown that yeast cells increase in size in the very early growth period and then decrease in size during the late exponential growth to a size comparable with that of the cells originally inoculated. The observations on protozoan populations have largely been made on

cultures contaminated by bacteria. Maupas in 1888 observed that individuals decreased in size during "senile degeneration" in old cultures. Calkins (1904) likewise showed that *Paramecium* decreased in size as the cultures died out. Woodruff's (1913) data on the growth of *Oxytricha fallax* can be roughly correlated with the growth phases. He observed that individuals were small during rapid growth and increased in size later. Peters (1921), on the other hand, noted that in cultures of *Colpidium colpoda* the population grew very slowly for nine to ten days during which time the individual size increased and remained large during the period of rapid growth which followed. Later the decrease in size was correlated with a decrease in population. Vieweger (1925) states that in cultures of *Colpidium colpoda* the body size decreased following the period of rapid exponential growth. His first measurements following the end of the exponential phase appear to have been made at six days, however.

All the work mentioned in the preceding paragraph was done with contaminated cultures and without complete data regarding the population curve being obtained. Bond (1933), using sterile cultures of *Tetrahymena geleii* (*Colpidium campylum*), and Lwoff (1932), using *Tetrahymena geleii* (*Glaucoma piriformis*), give data that indicate an increase in individual size following the exponential period. Their data concern total dry weight and total nitrogen change respectively. Dewey and Kidder (1940) give complete data on change in size in *Perispira ovum* fed on *Euglena gracilis*. They show an increase in size in the lag and early exponential phases. The size changes in *Perispira ovum* cannot be compared directly to those in *Tetrahymena geleii* since the increase in size in the former may be due largely to the accumulation of unassimilated *Euglena* in the individual ciliates. The subsequent decrease in size beginning in the late exponential period is correlated with a drop in the concentration of the food organism, *Euglena*. Any change in individual amount of *Perispira* protoplasm is obscured by the presence of masses of ingested but unassimilated *Euglena*.

The present investigations have brought out several points with regard to normal growth in populations of *Tetrahymena geleii*. The stationary phase of growth, so-called, has been found to be a truly stationary phase only so far as the numbers of individuals are concerned. It is seen that the number of fissions in this stage is very low while the population remains essentially static in number. This indicates that the individual organism has a much longer life in this phase and that there is very little death occurring. It is also seen that another process, that of individual growth, proceeds concurrently with that of population

growth until the end of the exponential phase whereupon it continues with the result that in the hours immediately following the onset of the stationary phase the organisms increase in size. It is thus apparent that the population curve taken alone is not a true representation of the growth of the culture.

Normal respiration data on *Tetrahymena geleii* is summarized in Table 5. M. Lwoff (1934) obtained the figure of 35 cu. mm. O_2/hr./mgm. dried organisms in peptone water using the Warburg method but does

TABLE 5

Comparative values of Q_{O_2} for Tetrahymena geleii.

Investigator	Approx. age of culture	Bacteria-free	Temp. °C.	Sub-strate pres-ent	Cu. mm. O_2/hr./10^6 organ-isms	Cu. mm. O_2/hr./mgm.
Peters (1929)[a]	?	Yes(?)	?	+	50–200	6.2–24.7[b]
M. Lwoff (1932)	?	Yes	22°	+	271.5[b]	35
Baker & Baumberger (1941)	48 hrs.	Yes	20°–22°	+	154[b]	19.01
Pitts (1932)	48 hrs.	No	24°	−	151	18.7[b]
R. H. Hall (1938–1941)	48 hrs.	Yes	19.8°	+	112.5	13.9[b]
Present investigator	50 hrs.	Yes	26.8°	−	133.7	16.5
Present investigator	50 hrs.	Yes	26.8°	+	632.5	77.7

[a] The ciliate used was called *Colpidium colpoda*. It may or may not have been *T. geleii*.

[b] These values are computed on the basis of the weight curve in Figure 2. 10^6 organisms equal 8.1 mgm. at 50 hrs.

not mention the age of the organisms used or the number of organisms per mgm. of dry weight. Pitts' (1932) figure was obtained using a non-nutrient suspension that had grown on bacteria for 48 hours previously. Baker and Baumberger (1941) used a dropping mercury electrode and a suspension of *Tetrahymena geleii* in yeast autolysate dextrose. They did not determine the dry weight but calculated it on the basis of 50 μ being the average length of an organism and the dry weight being about 15 per cent of the wet weight. R. H. Hall (1938, 1941) apparently used M/15 Sørensen phosphate and 0.5 M Clark and Lubs biphthalate buffers for his suspensions. It was found in the course of these investigations that M/50 Sørensen phosphate buffer depressed the respiration rate markedly and that even M/100 phosphate had a slight inhibitory effect.

A number of factors may have contributed to the different results shown in Table 5. The organisms in the several cases may not have been of comparable age and weight and there may have been consider-

able strain differences. Likewise there may have been some errors due to bacteria or the products of bacterial metabolism.

The age of 48 hours is significant only when the length of the exponential phase is known likewise, so the figures given in Table 5 on the age of the cultures are for the most part only rough approximations of the actual age of the ciliates in terms of the population curve. Strain differences are known to exist and may exert an influence on the rate of respiratory activity. The possible toxic or beneficial action of bacteria is well known. There may have been errors in the determinations by Baker and Baumberger due to their approximations regarding volume and weight of the ciliate. The inhibitory action of phosphate buffer solutions previously mentioned may vitiate Hall's figure. The different substrates used in the various determinations are undoubtedly influencing factors. The most probable reason for the high Q_{O_2} obtained in the present investigation is the utilization of a nontoxic concentration of buffer, and a substrate that is comparatively concentrated and to which the organisms have been thoroughly acclimated.

In comparing the rate of respiration in exponential and stationary phase ciliates in buffer suspensions on the basis of number of individuals, the values obtained were 140.2 cu. mm./hr./10^6 organisms and 133.7 cu. mm./hr./10^6 organisms respectively. When buffered 2 per cent proteose peptone suspensions were used the rate per 10^6 organisms is higher in the *stationary* phase. This situation is clarified when reference is made to Figure 2 and Table 4 and illustrates the errors that may be involved in comparing respiration rates on a population basis alone. The Q_{O_2} is defined as the cu. mm. O_2/hr./mgm. dry weight of respiring material. On this basis the Q_{O_2} of exponential phase ciliates is 26.2 since the weight of 10^6 organisms is 5.33 mgm. As the weight of the stationary phase ciliates increases rapidly following the end of the exponential period, the weight of 10^6 organisms at 50 hours is 8.10 mgm. This gives a Q_{O_2} of 16.5 or 63 per cent of the rate found in the exponential phase. This emphasizes the point that respiration rates should be compared on the per-unit-of-protoplasm basis rather than on the basis of individual organisms. The relatively greater increase in Q_{O_2} in stationary phase organisms when in proteose peptone suspensions suggests that the conditions under which measurements are made are much more like those of stationary phase cultures than those of exponential phase cultures due to the high concentration of organisms. External conditions may be limiting factors in the respiration of exponential phase organisms under these conditions.

In the matter of decreasing oxygen consumption with increasing age of the culture there is unanimity. Peters (1929) reports a drop from a Q_{O_2} of 200 in growing cultures of *Colpidium colpoda* to one of 50 in old cultures. Wachendorff (1912) reports a like decrease in the respiration of *C. colpoda* within 30 days from the time the culture was started. Baker and Baumberger (1941) show a decrease in *Tetrahymena geleii* suspensions beginning in the last exponential phase and dropping to approximately one-third the highest value obtained during that period, in from three to seven days. Hutchens (1941b) has found the same phenomenon in *Chilomonas paramecium* within a period of 72 hours. In the present investigations, likewise, a decrease in oxygen consumption has been found in the population phases following the exponential period of growth.

Summary

1. The relation of cell growth to population growth in bacteria-free cultures of *Tetrahymena geleii* has been examined.

2. Cell growth has been found to proceed independently after the onset of the stationary period of population growth.

3. The ratio of fissions occurring to the population concentration has been determined and described.

4. The normal Q_{O_2} of exponential and stationary phase ciliates in phosphate buffer and in proteose peptone has been determined.

5. A significant difference in the respiratory rates of organisms from the two phases has been shown.

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